**Results:** In SLE patients respect to HC, plasma IL-1β levels were unmodified whereas IL-6 was higher, resulting significantly increased in SLE-S. Mononuclear cells from SLE patients released lower quantities of IL-1β after stimulation with BzATP, whereas the release of both IL-6 and TNF-α was significantly augmented in SLE-NS respect to both HC and SLE-S subjects after all types of stimulation. RT-PCR showed reduced P2X7R and augmented NLRP3 mRNA expression in SLE patients. Accordingly, P2X7R activity was significantly reduced in all SLE patients and did not appear to be influenced by a chloroquine pre-treatment. Conclusion: In SLE patients, compared to HC subjects, we found reduced P2X7R mRNA expression, increased NLRP3 mRNA, as a possible compensating mechanism, and correspondingly, significantly lower BzATP-induced intracellular Ca²⁺ influx, without an apparent influence of a chloroquine pre-treatment.

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**References:**


**Disclosure of Interests:** Anna Lisa Gisolfi: None declared, Federica Furini: None declared, Alessandra Bortoluzzi: None declared, Marcello Govoni: None declared, Francesco Di Virgilio: Consultant for: FDV is a member of the Scientific Advisory Board of Biosceptre Ltd, a UK-based biotech company involved in the development of P2X7R-targeted therapeutics.

**Disclosure of interests:** Pei Han, Amy Meng, Nevena Mollova, Yuanjiang Yu, Julie A. Di Paolo. Gilead Sciences, Inc., Foster City, United States of America

**Background:** Inhibition of the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway has demonstrated efficacy in immune-mediated diseases and has been identified as a therapeutic target for the treatment of rheumatoid arthritis (RA). Differences in JAK inhibitor specificity for JAK1, JAK2, JAK3, and TYK2 may influence their safety profiles, but the mechanism is not known. Selective JAK1 inhibition by filgotinib (FIL) may modulate a subset of proinflammatory cytokines associated with RA pathogenesis and improve the risk-benefit profile by minimizing other non-JAK1-related adverse events. JAK2 inhibition is associated with myelosuppression, while JAK3 inhibition has been associated with increased risk for opportunistic infections (eg, tuberculosis and herpes zoster) and chronic low-grade inflammation. In clinical trials, FIL did not negatively impact hemoglobin, LDL/HDL ratios, or natural killer (NK) cell counts.1,2

**Objectives:** To compare the in vitro profile of JAK inhibitors with different JAK selectivity profiles, for effects on erythroid progenitor cell expansion, NK cell proliferation, and liver X receptor (LXR) agonist-induced cholesterol ester transfer protein (CETP) expression, an enzyme responsible for the conversion of HDL to LDL.

**Methods:** JAK inhibitors (FIL, FIL metabolite [GS-829845], baricitinib [BARI], tofacitinib [TOFA], and upadacitinib [UPA]) were evaluated in vitro in human cell-based assays: growth of erythroid progenitors from human cord blood CD34⁺ cells using a HemaTox™ liquid expansion assay, IL-15-induced NK cell proliferation, and LXR agonist-induced CETP expression in the hepatic cell line (HepG2). Using IC₅₀s generated from these assays and the reported human plasma concentrations of the JAK inhibitors from clinical studies,3,4 we calculated the target coverage for each compound at clinically relevant doses. The activity of FIL in humans was based on a PK-PD modeling algorithm5 of FIL + GS-829845.

**Results:** In vitro assay results are described in the table. Based on these results, human exposure data, and modeled PK-PD relationships, FIL 100 mg and FIL 200 mg result in lower calculated cellular inhibition than the other JAK inhibitors at clinical exposures. Notably, FIL 100 mg and FIL 200 mg, but not the other inhibitors, are calculated to reduce CETP expression by 17% and 27%, respectively, while BARI, TOFA, and UPA are not expected to alter CETP levels.

**Abstract THU0017 Table 1. IC₅₀ ± SD in vitro assays (nM, unless otherwise noted).**

<table>
<thead>
<tr>
<th>Assay</th>
<th>FIL</th>
<th>GS-829845</th>
<th>BARI</th>
<th>TOFA</th>
<th>UPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early erythroid progenitors</td>
<td>1960±157</td>
<td>19300±1730</td>
<td>&lt;150</td>
<td>&lt;150</td>
<td>&lt;150</td>
</tr>
<tr>
<td>Mature erythroid progenitors</td>
<td>1140±112</td>
<td>10600±1270</td>
<td>25.7±2.9</td>
<td>110±30</td>
<td>14.5±2.75</td>
</tr>
<tr>
<td>NK cell proliferation</td>
<td>314.8±53</td>
<td>9697±1000</td>
<td>6.6±1.9</td>
<td>12.2±2.1</td>
<td>4.1±1.7</td>
</tr>
<tr>
<td>Inhibition of LXR agonist-induced CETP expression</td>
<td>&gt;15.3±15.3</td>
<td>&gt;19.4±19.4</td>
<td>&gt;1 µM weak</td>
<td>No effect</td>
<td>±7.1 µM ±4.2 µM induction</td>
</tr>
</tbody>
</table>

3 Weak stimulation of LXR agonist-induced CETP expression.

**Conclusion:** JAK1 selectivity of FIL and GS-829845 resulted in less inhibition of erythroid progenitor expansion and NK cell proliferation compared with BARI, TOFA, and UPA. FIL also reduced LXR agonist-induced CETP expression, while the other inhibitors did not alter these levels. These results provide a potential mechanistic link to the observed reduction of CETP concentration and activity following FIL treatment, and the observed reduction in LDL/HDL in RA patients.6

**References:**


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**THU0018**

**ANTI-GALECTIN-9 ANTIBODY AS A NOVEL TREATMENT OPTION IN RHEUMATOID ARTHRITIS TARGETING PATHOGENIC FIBROBLAST-LIKE SYNOVICTES**

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**Background:** Fibroblasts-like synoviocytes (FLS) present in the stromal environment are key effector cells in the persistence of synovial inflammation and joint damage in rheumatoid arthritis (RA). A particular subset of FLS characterized as Thy-1+Podoplanin+CD34- is significantly expanded in RA patients and has been described to be disease-associated.1 Currenty, no treatment targeting the stromal environment in RA is available.2

**Objectives:** To identify a novel treatment option targeting the stromal environment in RA to modify the disease-associated subset of fibroblasts.3

**Methods:** An in vitro model was established with FLS derived from synovial fluid mononuclear cells (SFMC) from RA and osteoarthritis (OA) patients (n=6). FLS between passage 2-5 were used for further analyses. Untreated FLS in cultures were analyzed by flow cytometry for expression...
of the surface proteins CD34, CD45, Thy-1 and Podoplanin. The potential effect of cell detachment solutions on the expression of the surface proteins was examined by applying either trypsin or lidocaine. To evaluate on the inflammation status of the FLS, MCP-1 levels in supernatants from FLS in mono-cultures stimulated with either antibodies targeting galectin-9 (Gal-9), an anti-Gal-9 antibody-matched isotype control, LPS or steroid were analyzed by ELISA and compared to culture medium alone. Cell viability were examined using an MTT assay. Data are expressed as mean (95% CI) and analyzed by a paired t-test. P-values < 0.05 were considered statistically significant.

Results: FLS derived from SFMC were characterized as CD34 CD45 and the majority co-expressed Thy-1 and Podoplanin (80.5%, (66.3-94.7%)) confirming the pathogenic phenotype of these cells. This phenotype was not altered by using different methods to detach the cells from the cell-culture plates. FLS receiving anti-Gal-9 antibody treatment showed a significant decrease in MCP-1 secretion 0.84, (0.70-0.98) compared with unstimulated cells (p = 0.036). Treatment with an isotype control did not result in a significant decrease in MCP-1 secretion. This tendency was specific to FLS derived from RA patients as FLS derived from OA patients showed no significant decrease in MCP-1 secretion upon anti-Gal-9 antibody treatment. FLS derived from both RA or OA patients showed a significant increased fold change in secretion of MCP-1 upon LPS stimulation and significantly decreased levels of MCP-1 upon steroid treatment, consistent with the pathogenic phenotype of these cells. None of the different stimulations resulted in morphological changes of the FLS examined by light microscopy. Further, no significant changes in cell viability were detected after anti-Gal-9 antibody treatment.

Conclusion: FLS cultures derived from RA patients at passage 2-5 consist mainly of disease-associated fibroblasts and secrete significantly lower amounts of MCP-1 when treated with an anti-Gal-9 antibody without affecting cell viability. Thus suggesting that Gal-9 neutralization may represent a novel treatment option targeting the stromal environment and inflammation in RA.

REFERENCES:


Background: Systemic sclerosis (SSc) is an autoimmune disease ending in multorgan fibrosis. Vascular damage, responsible for the vascular alteration and disruption, has been suggested to play a key role in disease maintenance and progression [1]. Recent data demonstrated that high serum levels of the interferon (IFN)-γ-induced protein 10 (IP-10) in SSc patients correlated with peripheral vascular injury and increased in association with nailfold capillarscopic pattern worsening and digital ulcers presence [2, 3]. Between the vasoactive drugs used for SSc treatment, the prostacyclin analogue iloprost (I) and PDE-S inhibitor sildenafil (S) seem to have high vasodilatatory and immunomodulatory actions [4-6].

Objectives: To investigate and compare the ability of S and I to modulate: IP-10 circulating levels in SSc patients under different treatments; IP-10 release by human endothelial (Hfaec) cells subjected to Th1-related inflammatory stimuli.

Methods: Sera of 28 patients satisfying ACR/EULAR 2013 classification criteria for SSc were analyzed by ELISA. IFNy+TNFα-induced activation of NFkB, STAT1, JNK, ERK1/2 and AKT in Hfaec after S or I was taken as 100% (n=10, p<0.05, respectively). Between the vasoactive drugs used for SSc treatment, the prostacyclin analogue iloprost (I) and PDE-S inhibitor sildenafil (S) seem to have high vasodilatatory and immunomodulatory actions [4-6].

Results: The treatment with S significantly reduced IP-10 serum levels vs. with treatment (DMARDS) and corticosteroids (CCs) (184.1±65.10 to 880.9±339.0 pg/ml and vs. 426.5±101.7, respectively, P<0.01); while no significant difference has been found vs. I (184.1±65.10 to 282.7±46.6 pg/ml). In Hfaec, S and I differently counteracted the IFNγ+TNFα-induced phosphorylation of JNKs (respectively 61.0±20.1% and 95±13.1% of phosphorylation vs. I+T-induced taken as 100%), STAT1 (respectively 49.2±15.8% and 93.4±1.2% of phosphorylation vs. I+T-induced taken as 100%), NFkB (respectively 72.6±8.3% and 92±2.33% of phosphorylation vs. I+T-induced taken as 100%), ERK1/2 (respectively 31.6±7.9% and 27.2±4.4% of phosphorylation vs. I+T-induced taken as 100%), and AKT respectively 85.7±5.6% and 3.8±7.4% of phosphorylation vs. I+T-induced taken as 100%).

Conclusion: In vivo results show that S and I have more effect in targeting serum IP-10 in SSc patients than other therapeutic treatments. In vitro data show different inhibitory drug-induced effects on pathways underlying IP-10 production, depending on intracellular targets. S could be a potential pharmacological tool as effective as I to control IP-10 in blood or at endothelial cell level in SSc.

REFERENCES: